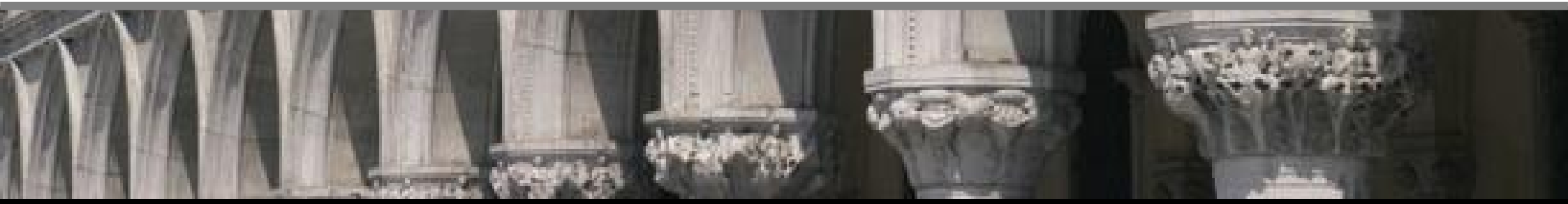
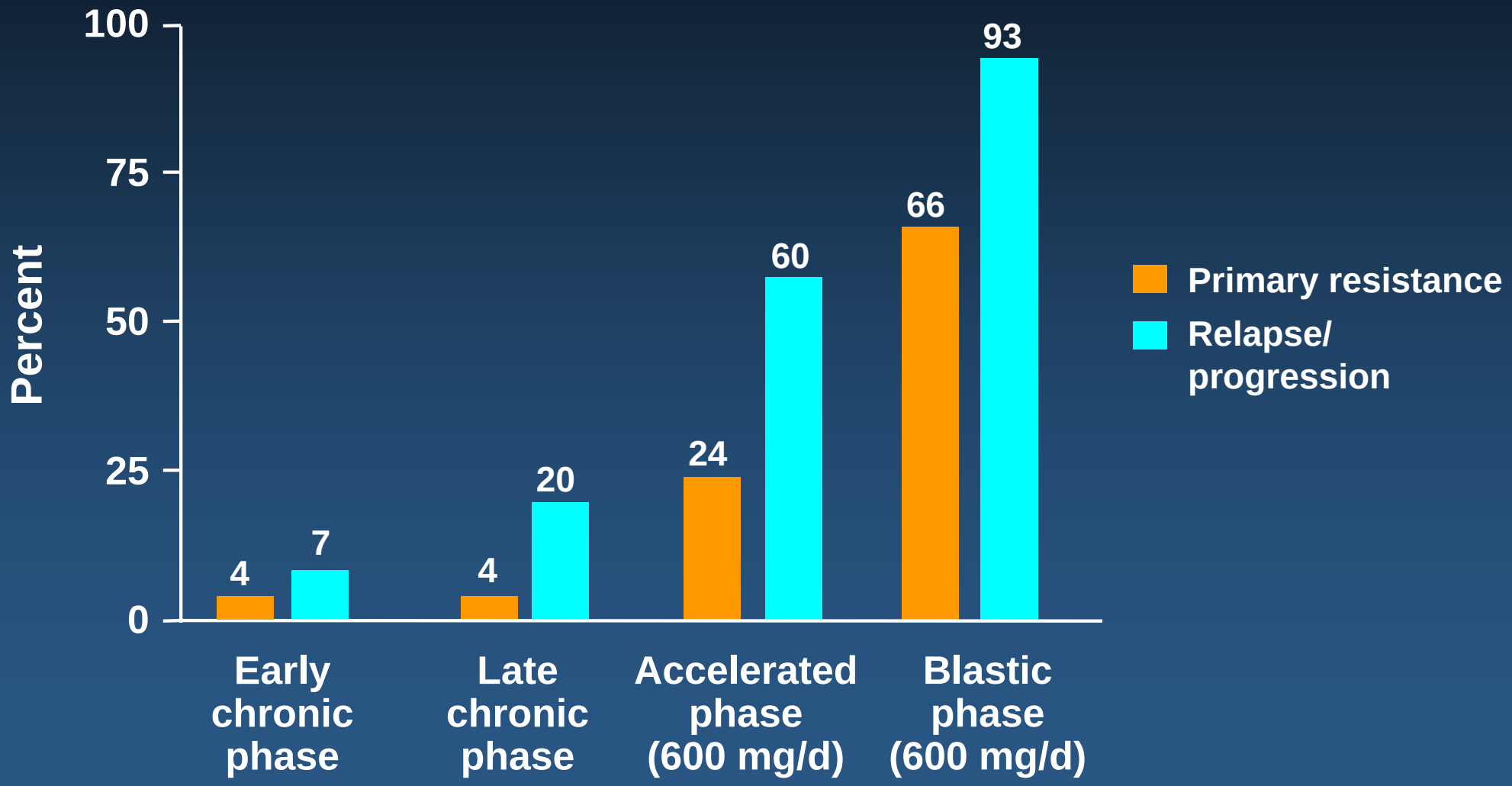
ELN European LeukemiaNet

Bcr-Abl Kinase Domain Mutations

Martin Müller



Frequency of imatinib resistance within 3 years



Hochhaus and La Rosée *Leukemia. 2004*

Observations associated with resistance

Genomic amplification / overexpression

BCR-ABL Mutations

Clonal evolution / aneuploidy

Pharmacologic mechanisms

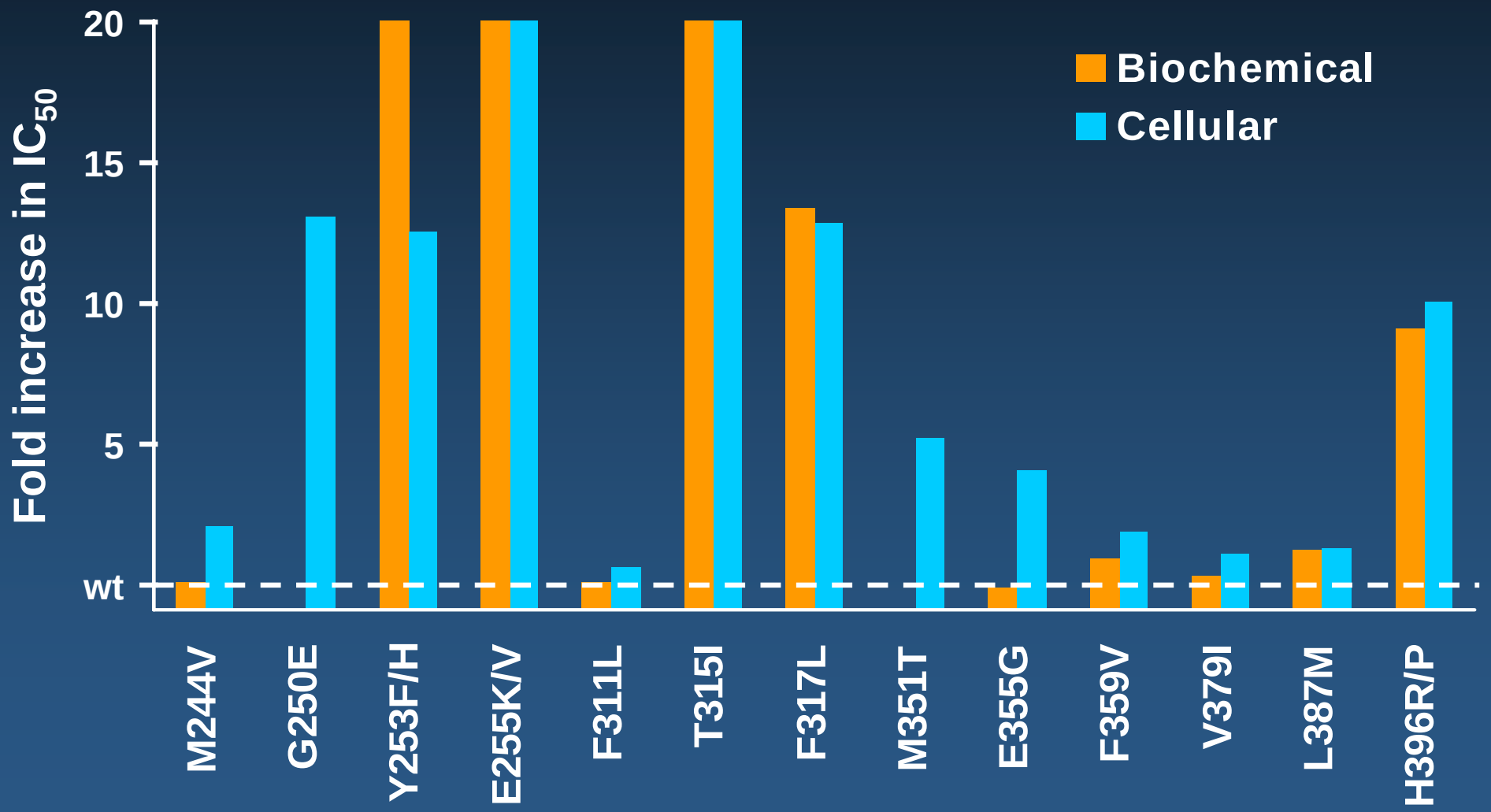
- Association with AGP-levels?
- Low MRP-1 levels independent prognostic factor (Lange et al.)
- Decrease of intracellular imatinib-levels in PGP+ patients (Illmer et al.)

Molecular - cytogenetic causes of resistance

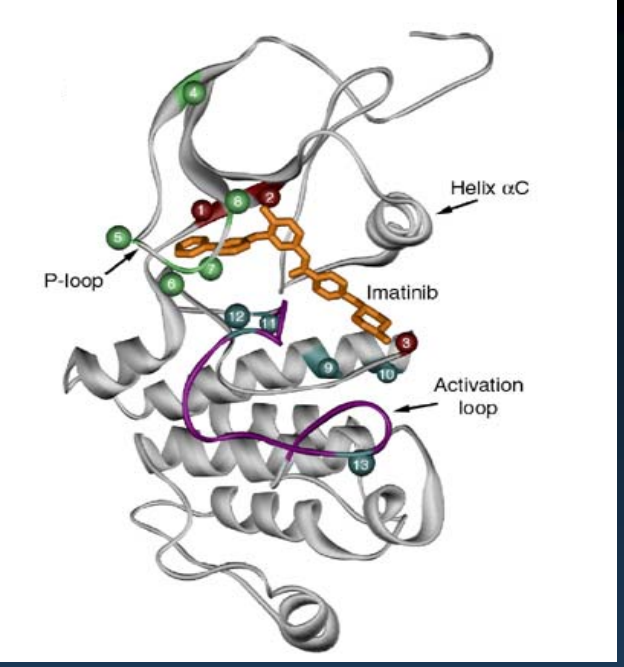
Patients With Hematologic Resistance/Relapse	Chronic phase (n=35)	Accelerated phase (n=33)	Blastic phase (n=66)	All (n=134)
***BCR-ABL* mutations (%)**	10/20 (50)	13/21 (62)	10/33 (30)	33/74 (45)
Clonal evolution (%)	15/29 (52)	8/16 (50)	16/22 (73)	39/67 (58)
Combination (%)	5/17 (29)	2/9 (22)	4/17 (24)	11/43 (26)

Lahaye et al. *Cancer. 2005*

Mutant BCR-ABL have increased IC_{50} values for imatinib mesylate



Corbin et al. *Blood.* 2003



Resistance to imatinib: 31 BCR-ABL mutations in 25 amino acids (n=126)

P-Loop | **Kinase domain** | **A-Loop**

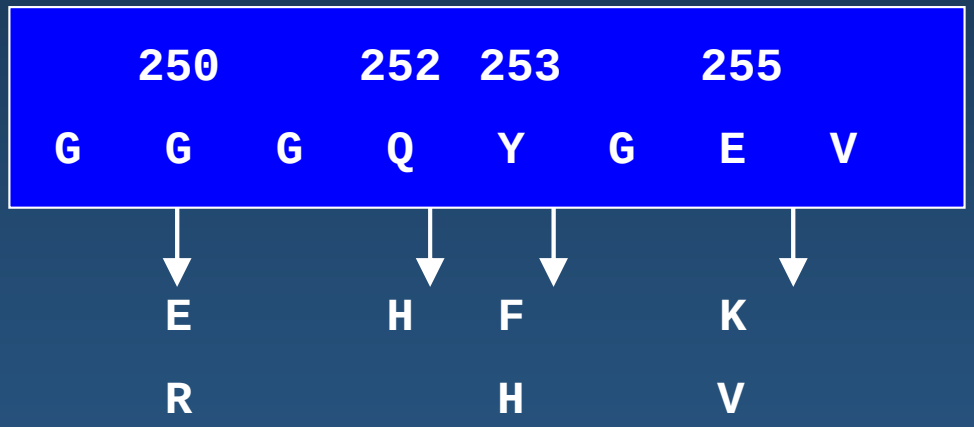
M244V **L248V** **G250E** **Q252H** **Y253F/H** **E255K/V** D267G T277A E279K F311L **T315I** F317L L324Q A344V A350V M351T E355G F359C/V V379I L387F/M M388L H396R A397P E450G/K E459K

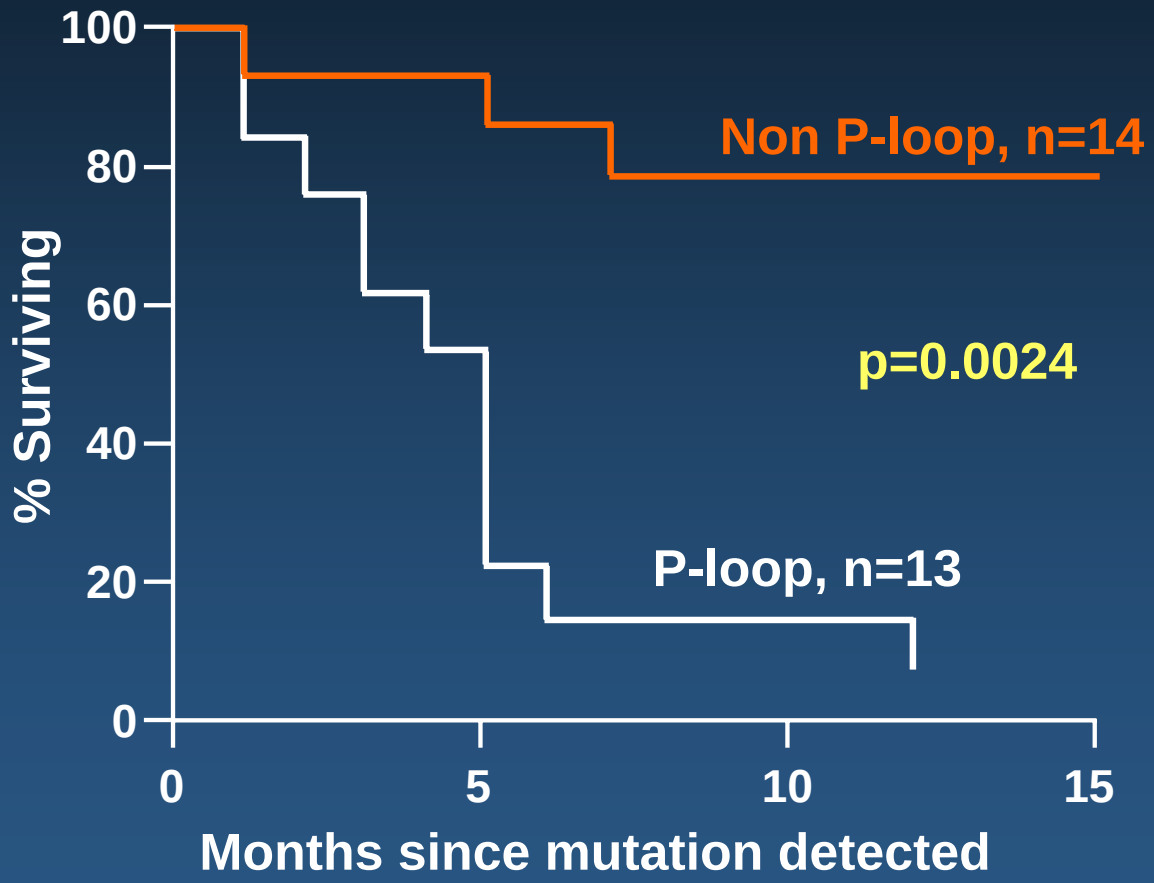
Polymorphisms: K247R T315T E499E

Survival after imatinib failure
Initial Australian experience

n=27

P-Loop: Highly conserved area

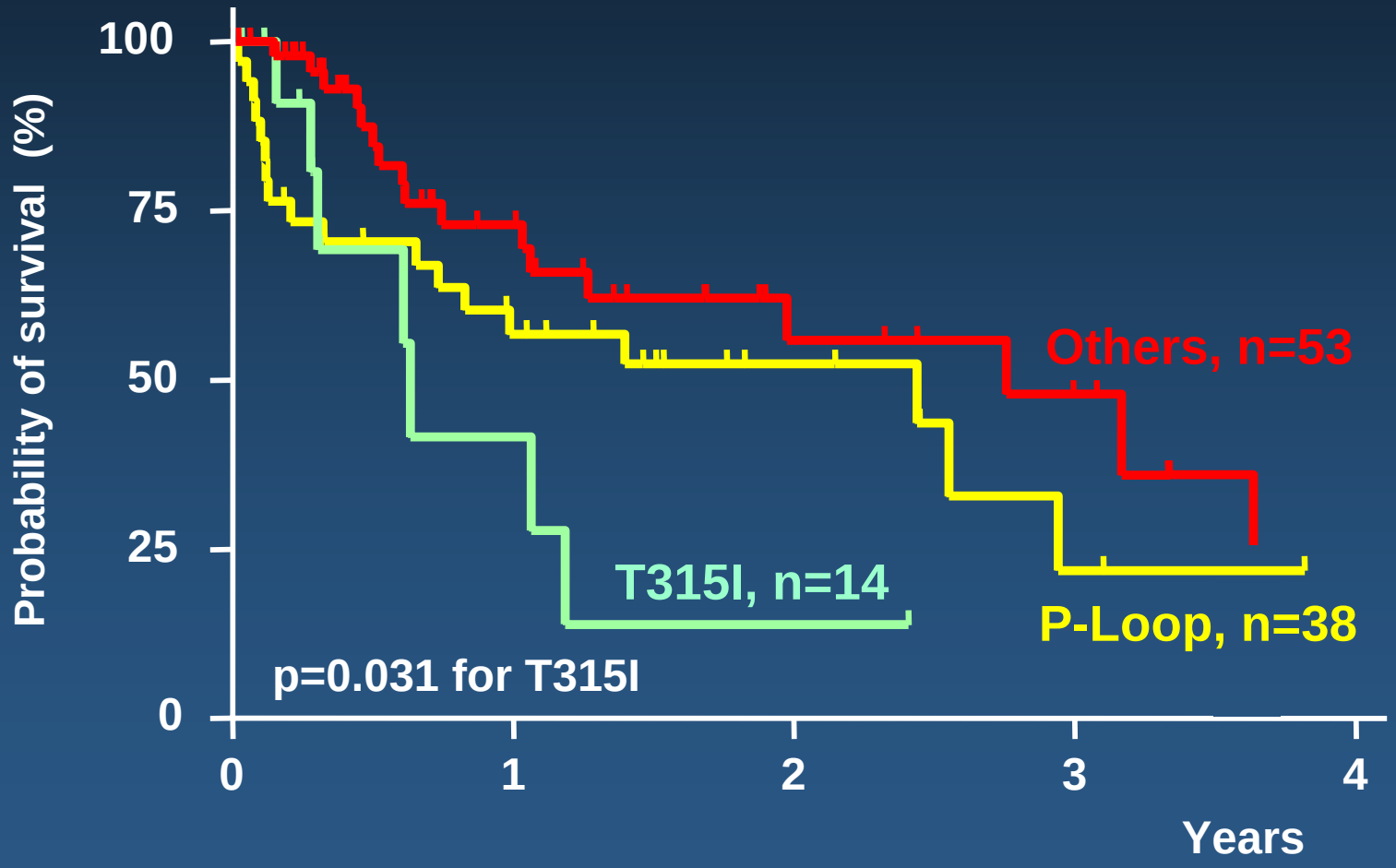




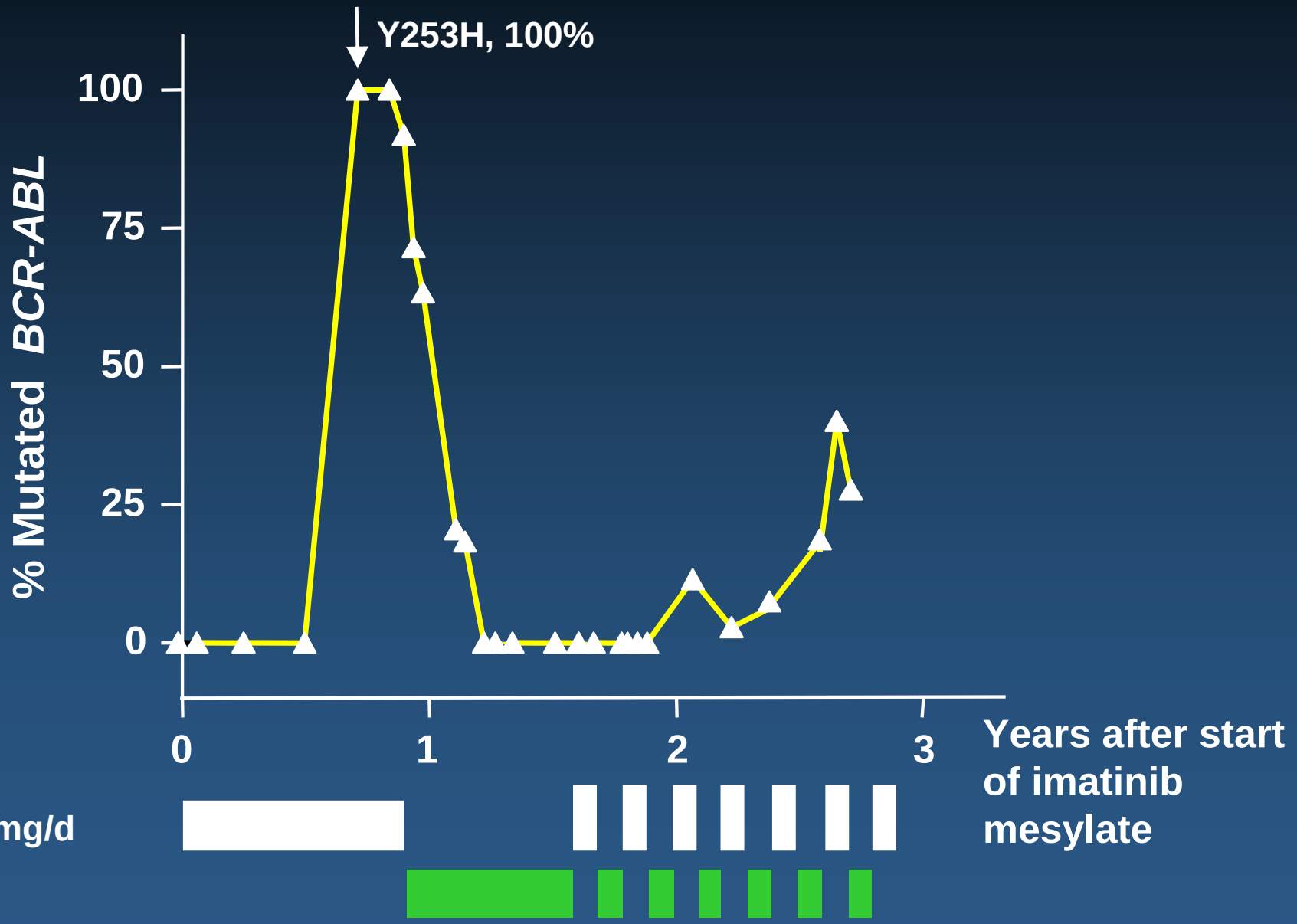
Branford et al. *Blood.* 2003.

Survival after imatinib failure
Current experience

n=105



CML, chronic phase, IFN-resistant



Significance of P-loop mutations in CML

- 89 imatinib resistant pts in 5 French centers;
  26 with P-loop mutations,
  18 with T315I, 50 with other mutations
  → significantly worse survival *from diagnosis*
  for P-loop or T315I mutations (p=0.014)

- 159 pts from MDACC:
  52 mutations in 49 pts; 19 P-loop mutations
  median F/U 6 mo:
  1/19 P-loop vs 4/30 non P-loop died;
  15 mo surv 95% vs 83%

Nicolini. *Leukemia.* 2006 Apr 27 [Epub ahead of print]
Jabbour. Blood 104; 288a, abst 1007, 2004

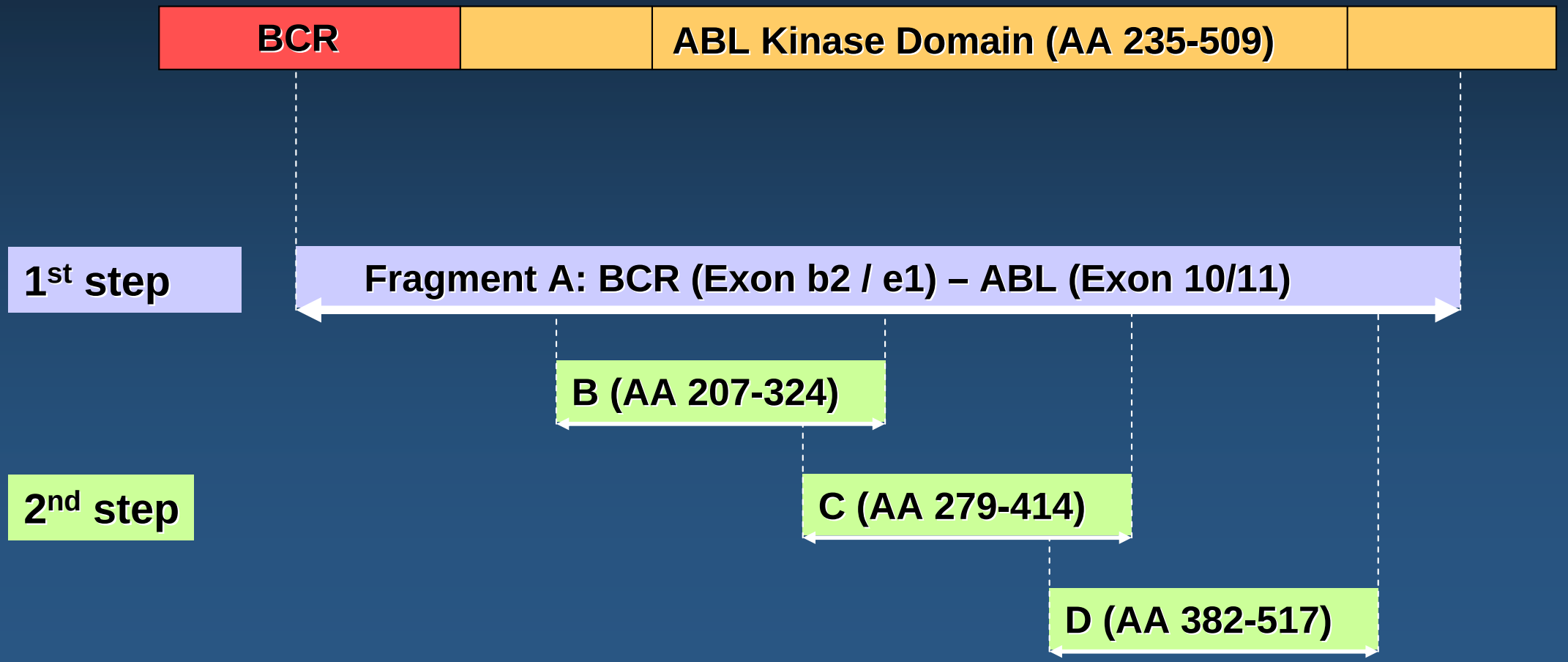
Indications for mutation screening

- hematologic resistance / relapse
- cytogenetic resistance / relapse
- 5-10-fold increase of BCR-ABL load
- prior to therapy with alternative kinase inhibitors (nilotinib, dasatinib)
- 3-monthly intervals under therapy with nilotinib and dasatinib

Methods to detect and quantify BCR-ABL mutations

	Specificity	Sensitivity
Sequencing	unspecific	10-20%
Restriction digest analysis	specific	~2-5%
D-HPLC	unspecific	0.1-10%
Allele specific PCR	specific	0.01%
Sequencing of clones	unspecific	1-5%

D-HPLC: nested RT-PCR



mutant BaF3^{T315I}

100%

50%

10%

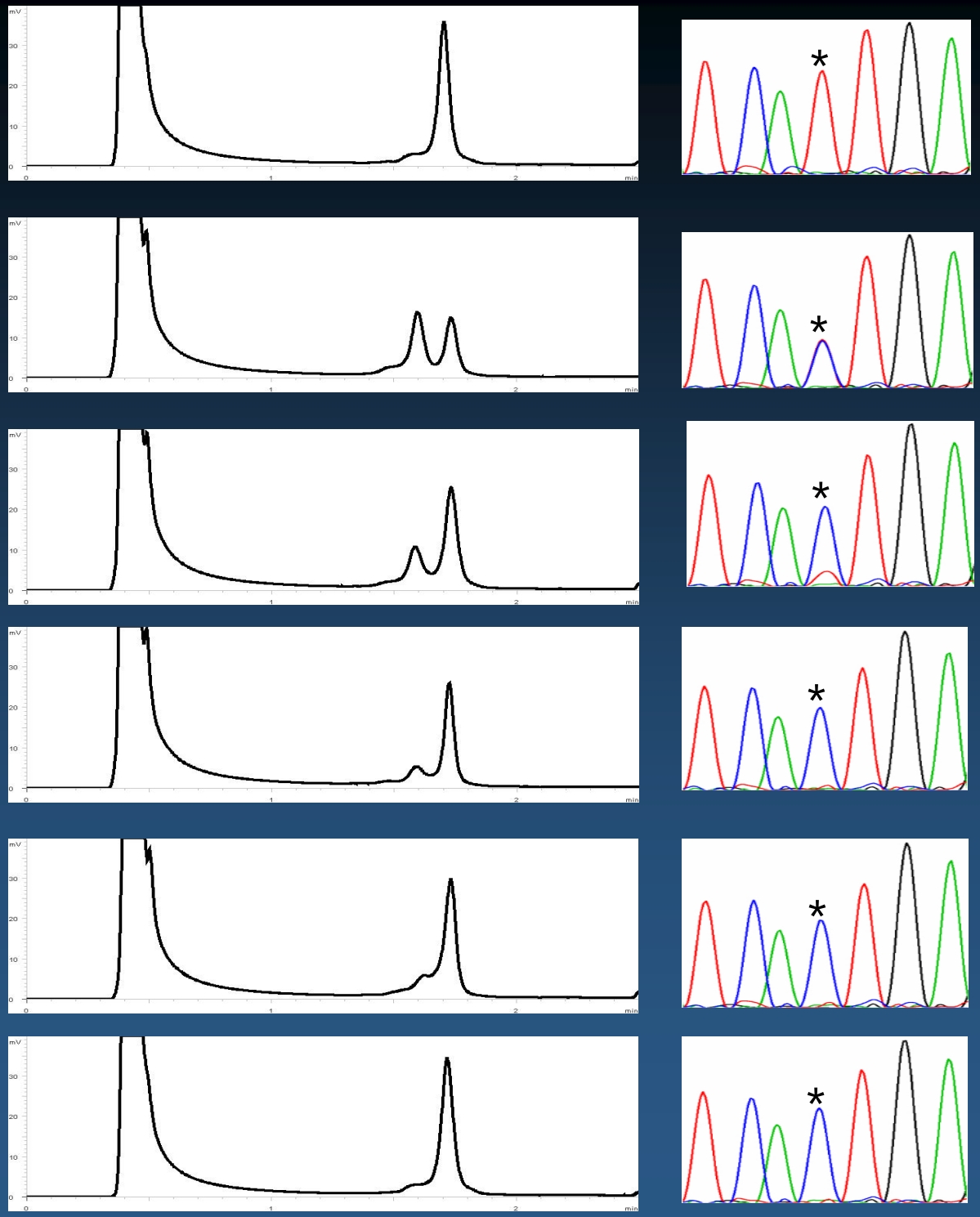
1%

0.1%

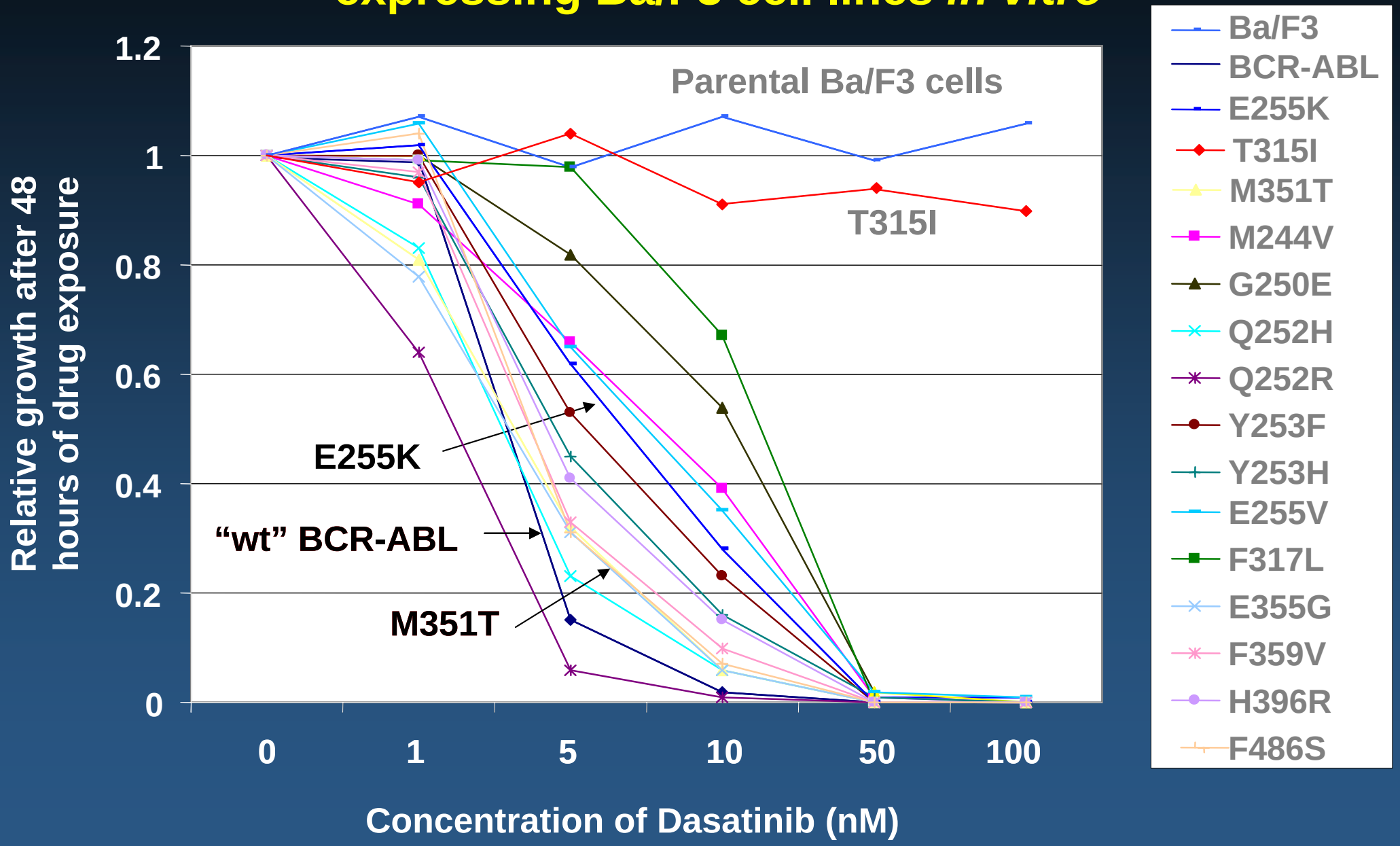
normal BaF3$^{BCR-ABL}$

Sensitivity of D-HPLC

0.1-1%



Dasatinib inhibits growth of 14/15 imatinib-resistant BCR-ABL-expressing Ba/F3 cell lines *in vitro*



Shah et al., *Science*, 2004

Conclusions

- Frequency of imatinib resistance depends on the stage of CML.
- The major causes of resistance are BCR-ABL mutations and clonal evolution.
- The impact of early detection of mutated clones by sensitive assays requires prospective evaluation.
- Certain BCR-ABL mutations might especially impair prognosis, in particular on continuous imatinib therapy.
- Early elucidation of imminent resistance to kinase inhibitors might contribute to individualized therapy according to molecular data.

EVOLVING CONCEPTS IN THE MANAGEMENT OF CHRONIC MYELOID LEUKEMIA

RECOMMENDATIONS FROM AN EXPERT PANEL ON BEHALF OF THE EUROPEAN LEUKEMIANET

EXIT